Supplement Contents

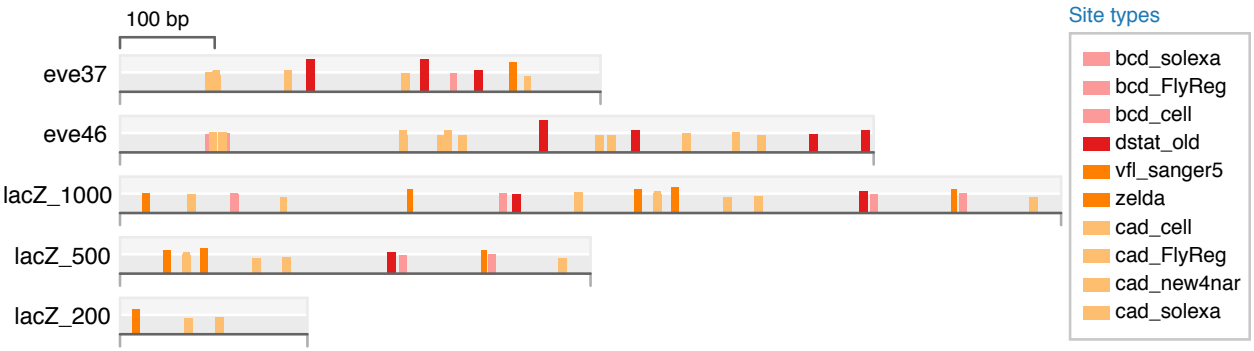

Figure S1: Activator binding sites in lacZ spacer sequence and enhancers. Predicted activator binding sites found using Patser with a cutoff of p=0.001 are plotted using InSite (see Materials and Methods). The ubiquitous activators dStat (red) and zelda (orange), as well as bcd (pink) and cad (light orange). Both enhancers have strong dStat sites, and eve 3/7 has one strong zelda site, but the activator binding sites are otherwise rather weak. An important note is that eve 3/7 does not have activator binding sites near either enhancer boundary (~90bp on the 5' and ~70bp on the 3' end)--suggesting that short range repressors would need to be very close to the enhancer boundary to influence them. Eve 4/6 also has a large buffer on the 5' end, but none on the 3' end. The 3' end is thought to be ~200bp longer than necessary for full 4/6 expression, although it's possible that those 200bp influence level. The lacZ spacers have quite a few weak activator binding sites. These might be expected to increase background expression, but would not drive spatially localized expression in the absence of repressors to restrict the expression domain.

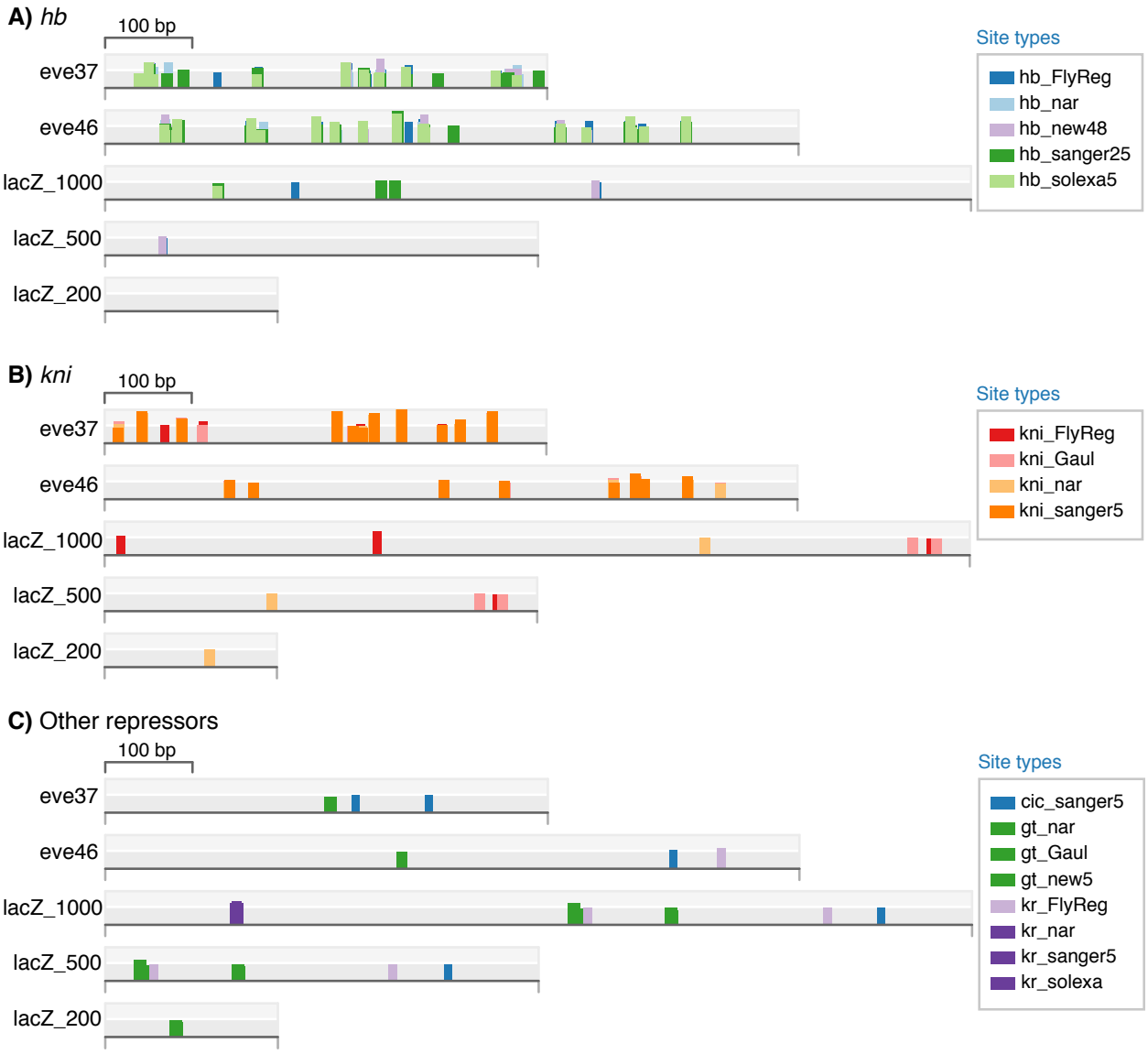

Figure S2: LacZ spacer sequences are depleted for known repressor binding sites. We first plotted the known 3/7 and 4/6 repressors *hb* (A) and *kni* (B) using multiple PWMs obtained from different types of experimental assays. As expected, eve 3/7 has many more and stronger *kni* binding sites than 4/6. However, it is not obvious that eve 4/6 is more sensitive to *hb* than 3/7. There are a few weak *hb* sites in the 1000bp spacer, but these are not in range to influence the enhancers (the closest to the boundary is ~125bp away). In the lacZ 500bp spacer, there is a single site (only predicted for some of the PWMs) which is close enough to influence the single dStat binding site at the 3' end of eve 4/6. The fact that eve 4/6 expression does not change based on orientation in the presence of the 500bp spacer argues against this site influencing expression (Figure 2). The spacers contain more predicted *kni* sites, although they are all of rather low affinity. One cluster is found on the 3' end of both the 1000bp and 500bp spacers. In the single enhancer controls, this cluster would be adjacent to the promoter and may be able to directly repress the promoter. The strongest argument against these sites being active is that the single enhancer controls each behave differently, rather than having consistent repression at the 500bp and 1000bp distances (Figure 2).

C) Other repressors: Capicua (blue) is a ubiquitous repressor. Other repressors that might influence the expression of the eve stripes are gt (green) and Kr (purple). One of the Kr PWMs predicts a number of low affinity sites (light purple), but all three of the other PWMs agree that there is a single moderately strong site in the lacZ 1000bp spacer. That site is 150bp from the boundary of the spacer, and hence unlikely to influence stripe expression. The only gt site with the potential to do anything is in lacZ_500 at the 5' end. This is the only repressor site that looks to be in range to potentially influence expression across the boundary.

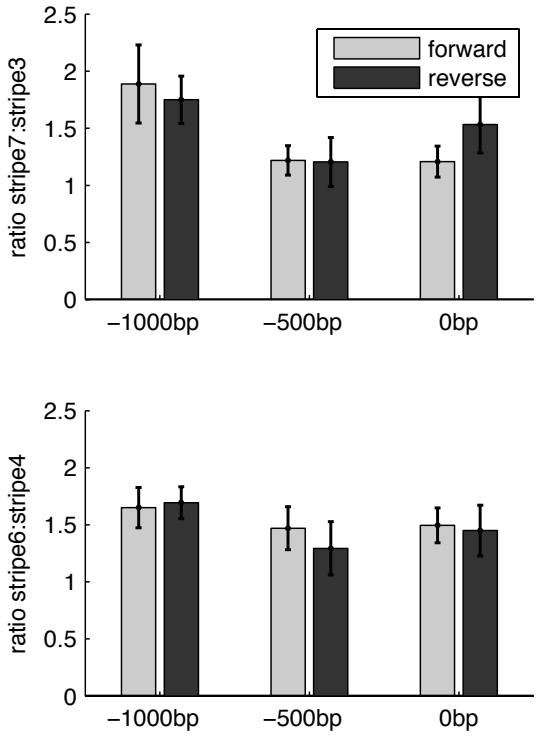

Figure S3: Distance and to a lesser extent orientation influence relative levels of expression driven in each stripe by a single enhancer. We measure mean expression in each stripe as in the fold-change plots. Top panel shows stripe 7 to stripe 3 ratios for each of the eve 3/7 constructs. Bottom panel shows stripe 6 to stripe 4 ratios for each of the eve 4/6 constructs. Error bars show 95% confidence interval of the mean.

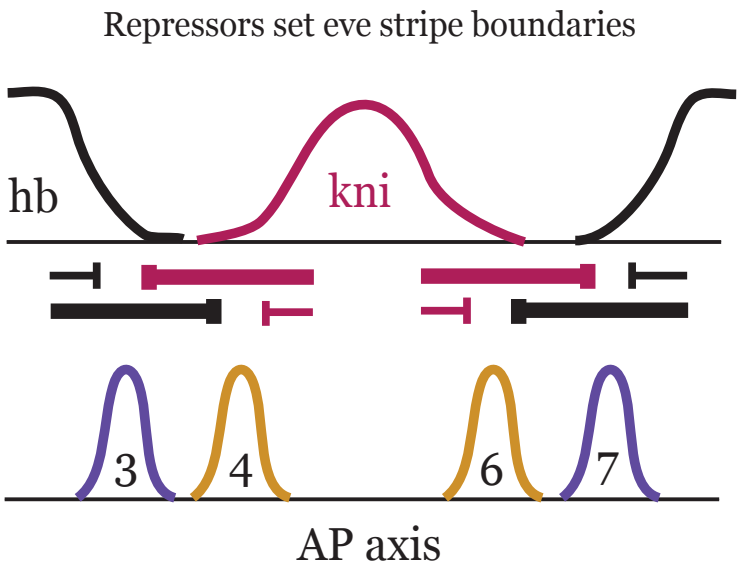

Figure S4: Regulation of stripes by *hb* and *kni*. The gap genes *hb* (black) and *kni* (magenta) set the boundaries of stripes in both eve 3/7 (blue) and eve 4/6 (yellow). Eve 3/7 is strongly repressed by *kni* and weakly repressed by *hb*, while eve 4/6 is weakly repressed by *kni* and strongly repressed by *hb*.

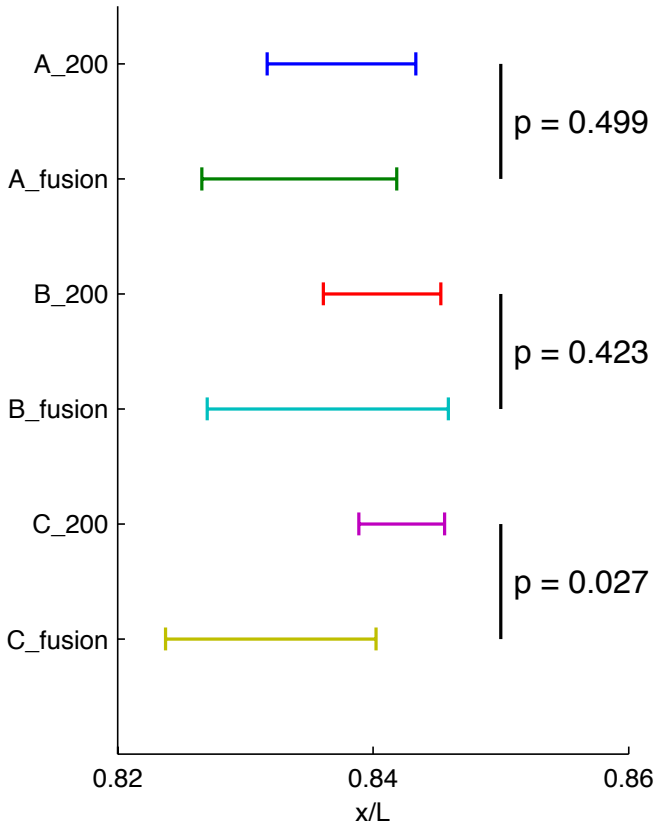

Figure S5: Stripe 7 peak position shifts slightly in fusions. We measured the AP position of stripe 7 peak expression (x/L) in individual embryos post-registration and plotted the 95% confidence interval of the mean (ie 1.96*SEM). Comparing between the 200bp configurations and fusions, we see an anterior expansion in the range of observed peaks, but only C_fusion is significantly different. After correcting for multiple hypothesis testing we find that p = 0.08, which suggests that this difference is not significant.

Table S1: Position weight matrices (PWMs) used in Supplemental Fig. S1 and S2 with sources.

TF	PWM Name	Reference
bicoid	bcd_solexa	Noyes et al. 2008a
bicoid	bcd_FlyReg	Bergman et al. 2005
bicoid	bcd_cell	Noyes et al. 2008a
bicoid	bcd_new5NAR	Noyes et al. 2008b
capicua	cic_sanger5	Zhu et al. 2011
caudal	cad_cell	Noyes et al. 2008a
caudal	cad_FlyReg	Bergman et al. 2005
caudal	cad_new4nar	Noyes et al. 2008b
caudal	cad_solexa	Noyes et al. 2008a
dStat	dstat	Noyes et al. 2008b
giant	gt_nar	Noyes et al. 2008b
giant	gt_Gaul	Schroeder et al. 2004
giant	gt_FlyReg	Bergman et al. 2005
giant	gt_new5	Noyes et al. 2008b
hunchback	hb_FlyReg	Bergman et al. 2005
hunchback	hb_nar	Noyes et al. 2008b
hunchback	hb_new48	Noyes et al. 2008b
hunchback	hb_sanger25	Zhu et al. 2011
hunchback	hb_solexa5	Zhu et al. 2011
knirps	kni_flyreg	Bergman et al. 2005
knirps	kni_Gaul	Schroeder et al. 2004
knirps	kni_NAR	Noyes et al. 2008b
knirps	kni_sanger5	Zhu et al. 2011
kruppel	kr_FlyReg	Bergman et al. 2005
kruppel	kr_NAR	Noyes et al. 2008b
kruppel	kr_sanger5	Zhu et al. 2011
kruppel	kr_solexa	Zhu et al. 2011
zelda	vfl_sanger5	Zhu et al. 2011
zelda	zelda	Satija and Bradley 2012

References

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